# SYSTEMATICS OF A NEW GENUS AND CAVERNICOLOUS SPECIES OF THE MOSQUITO TRIBE AEDINI (DIPTERA: CULICIDAE) FROM THAILAND 

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Abstract.-Borichinda Harbach and Rattanarithikul, n. gen., is introduced as a new mosquito genus of tribe Aedini for a previously unknown cave-dwelling species, Borichinda cavernicola Rattanarithikul and Harbach, n. sp., in Thailand. A diagnosis of the genus is provided that features unique anatomical characters of the adult, pupal, and larval stages of the type species. The affinities of Borichinda and Bc. cavernicola are discussed in terms of their position in the phylogeny of Aedini. The male and female genitalia, pupa, fourth-instar larva, and terminal abdominal segments of the third-instar larva of the new species are illustrated. Sequence data are provided for the second nuclear internal spacer region (ITS2) and a 522-bp fragment of the mitochondrial cytochrome c oxidase I (COI) gene. Cladistic analysis of morphological data indicates that Borichinda is more closely related to Isoaedes and Ayurakitia than to other genera of tribe Aedini. Salient differences that distinguish these three genera are contrasted.

Key Words: new genus, new species, ITS2, COI, mosquito, taxonomy, systematics

Larvae of the new species described in this paper were discovered in a cave while sampling a wide range of larval mosquito habitats in Doi Inthanon National Park in Chiangmai Province of northern Thailand. Adults reared from the larvae were initially identified as a species of genus Isoaedes (sensu Reinert et al. 2004) using unpublished keys (Rattanarithikul et al.) for the aedine fauna of Thailand. Upon closer examination of the adults and their associated larval and pupal exuviae, it became obvious that the species was very different from Isoaedes, suggesting that it belonged to a hitherto unrecognized
phyletic line of tribe Aedini. Consequently, character data for the new species were coded for the characters described by Reinert et al. (2004) and the combined data set analyzed to objectively assess the placement of the new species in the classification of the tribe. The results of this analysis indicate that the species does not fall within any currently recognized genus-level taxon of Aedini, and therefore a new genus-species combination is proposed and described herein.

## Materials and Methods

This study is based on larvae and adults reared individually from larvae
and pupae collected from a rimstone pool in a cave in northern Thailand (see Material examined following the species description). Pinned adults were examined under simulated natural light; dissected genitalia, larvae, and larval and pupal exuviae were studied with differential interference contrast optics. Measurements and counts were taken from 10 specimens of each life stage. Numbers in parentheses represent modes of the reported ranges. Anatomical terminology and abbreviations used in the descriptions and illustrations, respectively, follow Harbach and Knight (1980, 1982). The symbols o, $^{\hat{0}}$, Le, $\mathrm{Pe}, \mathrm{L}^{4}$, and $\mathrm{L}^{3}$ used in the material examined section represent female, male, larval exuviae, pupal exuviae, fourth-instar larva, and third-instar larvae, respectively.

A number of fourth-instar larvae were killed and preserved in $95 \%$ ethanol for DNA extraction. DNA was extracted from three larvae using the commercially available QIAgen DNAeasy Kit (QIAgen Ltd, Sussex, England) following the manufacturer's recommended protocol. A 522-bp fragment of the mitochondrial cytochrome oxidase subunit I locus (COI) was amplified using the universal insect primers C1-J-1718 and C1-N-2191 (Simon et al. 1994). The last five digits of these codes indicate the position and orientation of the $3^{\prime}$ end of the primers with respect to the mtDNA genome of Drosophila yakuba (X03240) (Clary and Wolstenholme 1985). Amplification of the 393-bp amplicon of the second nuclear ribosomal spacer region (ITS2), including flanking portions of the 5.8 S and 28 S genes, was carried out using the 5.8SF and 28SR primers recommended by Collins and Paskewitz (1996). PCR products were amplified using the following reaction mix $(50 \mu \mathrm{l}): 2 \mu \mathrm{l}$ DNA, $25.5 \mu \mathrm{ld} \mathrm{H}_{2} \mathrm{O}, 2.5 \mu 12.5 \mathrm{mM} \mathrm{MgCl}_{2}$ (BioLine, London, England), $0.1 \mu \mathrm{l}$ Taq polymerase (BioLine), and $5 \mu 1$ each of primers at $5 \mu \mathrm{M}, 2 \mathrm{mM}$ dNTPs (PE

Applied Biosystems, Warrington, England), and $10 \times \mathrm{NH}_{4}$ buffer (BioLine). The PCR thermocycler program consisted of a 2 -min denaturation at $94^{\circ} \mathrm{C}$, 34 cycles at $94^{\circ} \mathrm{C}, 53^{\circ} \mathrm{C}\left(\right.$ ITS-2) $/ 57^{\circ} \mathrm{C}$ (COI), and $72^{\circ} \mathrm{C}$ for 30 sec each, followed by a $10-$ min extension at $72^{\circ} \mathrm{C}$. Sequence data were obtained following PCR purification using a commercially available PCR purification kit (QIAgen Ltd, Sussex, England), and products diluted to $3 \mathrm{ng} / \mu \mathrm{L} / 200 \mathrm{bp}$ of product for sequencing. Cycle sequencing reactions were prepared using one-eighth reactions of the Big Dye Terminator Kit (PE Applied Biosystems) and read by an ABI 3730 robotic sequencer (PE Applied Biosystems). Sequence data were edited and aligned using Sequencher®(II) 4.5 (Genes Codes Corporation, Ann Arbor, Michigan). After sequencing, the DNA was dried and retained at $-70^{\circ} \mathrm{C}$ in the Molecular Systematics Laboratory, The Natural History Museum, for future reference.

The phylogenetic relationship of Borichinda to other aedine taxa was examined by including character data for Borichinda in the parsimony analysis of Reinert et al. (2004), which should be consulted for the methods describing the cladistic analysis. Briefly, 172 characters derived from eggs, fourth-instar larvae, pupae, and adult males and females (Appendix) were coded for 120 species: Borichinda cavernicola, n. sp., plus the 119 exemplar species representing the 12 genera and 56 subgenera of tribe Aedini that were recognized prior to the changes to aedine classification proposed by Reinert et al. The coding by Reinert et al. of character 38 for Isoaedes cavaticus was changed from state (1) to (2) (see Reinert 1979: fig. 5). The data were analyzed using both equal weights, implemented by WinClada version 1.0000 (Nixon 1999-2002), and implied weights, implemented by PIWE version 3.0 (for Windows) (Goloboff 1997) with the de-
fault value of the concavity constant, $K$ $=3$. The former applied the Parsimony Ratchet (Nixon 1999) (50,000 replications with 17 characters sampled and one tree held per replicate). The latter was performed by heuristic search, using 5,000 replications (mult*5,000) and holding 10 cladograms per replicate (hold/ 10). To determine whether the Ratchet and PIWE had found all most parsimonious or fittest cladograms (MPCs), respectively, the results were checked by searching for successively less parsimonious or fit cladograms using the commands "sub $n$ " (where $n$ is the increase in length in steps of 1 (equal weighting) or decrease in fit, in steps of 0.1 (implied weighting)) and "find*" (to search for all cladograms of length +n or best fit $-n$, respectively, up to a maximum of 100,000 cladograms. The "best" command was then applied to this set of 100,000 cladograms to confirm that the included set of MPCs was the same as that obtained in the initial analyses. Agreement in grouping within a set of MPCs was summarized using a strict consensus tree (SCT). A SCT of the combined EW and IW analyses was generated to reveal those taxa whose composition and relationships are weighting independent.

## Taxonomic Treatment

## Borichinda Harbach and Rattanarithikul, new genus

Type species: Borichinda cavernicola Rattanarithikul and Harbach, n. sp.

Adults.-Medium-sized mosquitoes. Moderately ornamented, principally dark-scaled with pale-scaled areas and markings; setae of head, antepronotum, scutum and scutellum strongly developed, prominent, long and dark; setae of thoracic pleura and coxae lighter in color and not so prominent. Head: Narrow pale decumbent scales and darker erect forked scales on occiput
and vertex; ocular line narrow, with sparse narrow pale scales; interocular scales extend between eyes to postfrons; eyes narrowly separated by space equal to diameter of 1-2 eye facets. Maxillary palpus of females with 3 palpomeres; palpus of males with 5 palpomeres, palpomeres 2 and 3 ankylosed. Thorax: Scutum with pattern of long narrow dark and pale falcate scales covering all but inner dorsocentral areas at anterior promontory and prescutellar area; setae as follow: complete acrostichal line, complete dorsocentral line contiguous with lateral prescutellar line, anterior, lateral, median and posterior areas of scutal fossa, antealar and supraalar areas; scutellum with broad pale spatulate scales on middle lobe and narrower scales on lateral lobes; paratergite and mesopostnotum bare. Antepronota widely separated, not enlarged, with cluster of 3-6 strong dorsally projecting setae on upper margin and scattered setae below. Postpronotum with variable number of pale falcate scales along dorsal margin. Pleura with small patches of pale spatulate scales on upper proepisternum, postspiracular and subspiracular areas, upper and lower mesokatepisternum and upper (anterior) mesepimeron. Wing: Dark-scaled; remigial setae absent; alula with row of narrow scales on margin; upper calypter with row of setae on dorsal half of margin. Legs: Dark-scaled, femora with narrow apical pale rings; fore- and midungues of females each with tooth, hindungues simple; posterior foreunguis of males large, with tooth, anterior foreunguis smaller, without tooth, midungues each with tooth, hindungues simple. Abdomen: Terga with basal pale bands that become obsolescent medially on posterior segments. Female genitalia: Tergum and sternum VIII with numerous broad spatulate scales; tergum VIII with setae on posterior 0.6, basolateral seta absent; sternum VIII with median
caudal emargination, seta 2-S noticeably posterior to seta $1-S$; tergum IX relatively narrow, width about $1.7 \times$ length, roughly cordate in outline, posterolateral corners with group of several fine setae; insula tonguelike, without setae; upper vaginal sclerite weakly developed (not illustrated); lower vaginal sclerite absent; spermathecal eminence membranous, poorly defined; postgenital lobe moderately long and wide, apex with relatively deep emargination, caudal half of ventral surface with scattered setae; cercus moderately long, scales absent; one large and 2 smaller spermathecal capsules. Male genitalia: Tergum IX with slightly produced lobe on either side of narrow median bridge, each lobe with cluster of relatively slender stiff setae; sternum IX long, with median posterior group of fine setae; gonocoxite long and relatively narrow, mesal surface entirely membranous; gonostylus attached at apex of gonocoxite, single gonostylar claw at apex; basal mesal lobe elongate, free of mesal membrane of gonocoxite; proctiger long, paraproct simple, without sternal arm; cercus membranous, setae absent; aedeagus moderately long, comprised of 2 lateral plates (aedeagal sclerites).

Pupae.-Cephalothorax: Seta 1-CT similar in development to 3-CT; 5-CT longer than $4-\mathrm{CT} ; 6,7-\mathrm{CT}$ subequal; 10,11-CT on small tubercles, closer to one another than to $12-\mathrm{CT}, 11 \mathrm{CT}$ single; 13-CT absent. Trumpet: Tracheoid present, weakly developed. Abdomen: Seta 6I,II longer than 7-I,II respectively; 2-IIVII inserted anterior and slightly mesad of setae 3 and 4, laterad of seta $1 ; 6$-II-VI long, single (infrequently double on II and III), inserted posterior to setae 4 and 9, 6-VII inserted anterior to seta 9; seta 9-II-VI comparatively long, single, inserted near midlength of lateral margin considerably anterior to seta $6,9-$ VII longer than 6-VII; 10-II present, long, single; 5-IV-VI long, usually single, longer than
following tergum; 4-V inserted on line directly mesad of seta 5,4 -VI in line with seta 5 ; 3-VI inserted lateral to $1-\mathrm{VI}$. Paddle: Longer than wide, outer part slightly broader than inner part; midrib indistinct distally; apex slightly emarginated; without marginal fringes, outer part with minute serrations proximal to midlength. Seta 1-Pa single, rarely distally forked; 2-Pa absent.

Larvae, fourth-instars.-Head: Median labral plate not apparent or absent. Occipital foramen more or less oval, longer in ventral view than posterior view, collar more strongly developed dorsolaterally. Hypostomal sclerite triangular, narrowly attached to lateralia. Labiogula short; hypostomal suture complete, gently curved, extending to posterior tentorial pit at margin of collar. Setae 5,6,8,9,10,13-C single, 9-C rarely double; 4-C with multiple thin branches, inserted much closer to 6-C than to $5-\mathrm{C} ; 7-\mathrm{C}$ inserted more or less on level with 4-C, far anterior to 5-C; 8,10-C inserted more or less at same level; 9-C inserted posterior to $8,10-\mathrm{C} ; 11,12-\mathrm{C}$ more or less equal distance from 13-C, $12-\mathrm{C}$ mesal to $13-\mathrm{C}, 13-\mathrm{C}$ in line with $11-$ C; 15-C relatively long, single, occasionally double; 19-C absent; ventromedian cervical sclerite present. Antenna: Long, slender, smooth, curved mesad; seta 1-A relatively long, normally double, borne dorsolaterally at midlength. Thorax: Setae $0,1,3,8,14-\mathrm{P}, \quad 1,13,14-\mathrm{M}$ and 1,5,8,13-T stellate; $0-\mathrm{P}$ more or less directly posterior to 4-P; 1-3-P not attached to common setal support plate; 2-P single, longer than 1,3,4-P; 13-P absent; $7-\mathrm{M}$ shorter than $5-\mathrm{M}$; 2-T single. Abdomen: Setae 1,2,5,9,13-I-IV, 7-II-VI, 11-I and 1,5-VIII well developed, stellate, minutely aciculate; 2-IVII inserted anterolateral to seta 1; 6-IVI long, branched aciculate; 3-I single; 7-I nearly as long as 6-I, with 2-4 branches; 12-I absent; 7-II much smaller than 6-II; 9-II-V inserted far anterior to
seta 7; 10-II-V slightly mesad of setae 11,12. Segment VIII: Comb with relatively large spinelike scales in single row. Seta 5-VIII noticeably ventral to comb. Siphon: Slightly swollen just beyond midlength; acus present, small, detached; pecten with evenly spaced spines; seta 1S inserted distal to pecten. Segment $X$ : Saddle incomplete, relatively large, extending below lateral midline of segment X , posterior margin lined with spinelike spicules, acus absent. Seta 1-X well developed, inserted on and longer than saddle; 3-X normally single, rarely double on one side; 4-X with 4 pairs of setae on grid (with only transverse bars) and 2 stellate precratal setae, cratal setae include 2 short normally single setae anteriorly and 6 long setae with $1-4$ branches posteriorly.

Eggs.-Unknown.
Etymology.-Borichinda is the name of the cave where the type species of the genus was discovered (see below). The cave bears the surname of Mr. Thanom Borichinda, the 11 th chief of Chomthong District (1927-1929) in Chiangmai Province, Thailand, where the cave is located. Hence, the gender of Borichinda is masculine in agreement with Article 30.2.2 of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999). The two-letter abbreviation $B c$. is recommended for this genus.

Systematics.-The generic status and phylogenetic relationships of Borichinda were assessed objectively by including character data for Borichinda in the parsimony analyses of Reinert et al. (2004). The characters used in the analyses and their states observed in Borichinda are listed in the Appendix. Analysis of the data set under equal weights (EW) produced 128 most parsimonious cladograms (MPCs) with a length of 1853 steps $(\mathrm{CI}=0.12, \mathrm{RI}=$ 0.65 ). Borichinda was placed in an unambiguous sister-group relationship with

Isoaedes in the strict consensus tree (SCT) of these cladograms. Analysis of the data under implied weights (IW) yielded 56 MPCs of fit 595.0 , in which Borichinda was placed in two alternative clades: Isoaedes + (Borichinda + Ayurakitia) and Isoaedes + Borichinda. The former clade occurred in 38 MPCs and the latter occurred in 18 MPCs. The sister relationship of Borichinda + Ayurakitia is supported by seven homoplastic characters (19:0, 33:1, 34:0, 36:1, 55:1, 137:1, 159:2) and that between Borichinda + Isoades is supported by seven different homoplastic characters (5:1, 45:1, 60:1, 68:0, 85:1, 103:0, 135:0). Three of the characters that support the sister relationship of Borichinda + Isoades also support Isoades as the sister of Borichinda + Ayurakitia (5:1, 68:0, 103:0). Borichinda emerged as a separate lineage in an unresolved basal polytomy along with 30 other taxa (see fig. 7 of Reinert et al. 2004) when the SCTs of the EW and IW analyses were combined. Incidentally, all the taxa that Reinert et al. (2004) treated as genera were recovered here as monophyletic groups, and groups comprising two or more genera exhibited identical or very similar patterns of interrelationships.

Borichinda clearly falls within the Aedes genus-group of Reinert et al. (2004), but is not consistently placed either within a genus or as sister to any of the genera recognized by those authors. Because Borichinda is paired with Isoaedes in both the EW and IW analyses, it could be argued that Borichinda might be considered a subgenus of Isoaedes. However, the autapomorphic differences of Borichinda in comparison to both Ayurakitia and Isoaedes are so great that it cannot be readily accommodated within either. The many salient differences between these three taxa that are not reflected in the data set used in the cladistic analyses are contrasted in Table 1. These differences

Table 1. Salient anatomical differences that distinguish the adults, pupae, and fourth-instar larvae of Borichinda, Isoades, and Ayurakitia. Characters that distinguish Borichinda from Isoaedes are indicated with an asterisk (*).

| Character | Borichinda | Isoaedes | Ayurakitia |
| :---: | :---: | :---: | :---: |
| Adults |  |  |  |
| Vertex, decumbent scales | Narrow | Narrow | Broad |
| Ocular scales | Narrow | Narrow | Broad |
| Compound eyes* | Narrowly separated | Continguous | Continguous |
| Interocular setae | Present | Present | Absent |
| Interocular space | Narrow | Narrow | Broad |
| Scutum, pale scaling* | Present | Absent | Absent |
| Scutellum, scales on midlobe* | Broad | Narrow | Narrow and broad |
| Antepronotal scales | Present | Present | Absent |
| Postpronotal scales | Present | Present | Absent |
| Postspiracular setae | Present | Present | Absent |
| Postspiracular scales* | Present | Absent | Absent |
| Subspiracular scales* | Present | Absent | Absent |
| Remigial setae* | Absent | Present | Present |
| Foreungues (males)* | One toothed | Both toothed | One toothed |
| Midungues (males) | Both toothed | Both toothed | Both simple |
| Female genitalia |  |  |  |
| Insular setae | Absent | Absent | Present |
| Male genitalia |  |  |  |
| Sternum IX (males)* | Long | Shorter | Long |
| Sternum IX setae* | Present | Absent | Present |
| Gonostylus | Long, cylindrical | Long, cylindrical | Short, bilobed |
| Claspette* | Apex expanded with numerous setae | Apex narrow with few setae | Apex narrow with few setae |
| Pupae |  |  |  |
| Seta 7-CT* | About length of 6-CT | Longer than 6-CT | Longer than 6-CT |
| Seta 3-III* | Single | Multiple branches | Single |
| Seta 6-III* | Single | Branched | Branched |
| Seta 6-VII* | Anterior to seta 9 | Posterior to seta 9 | Posterior to seta 9 |
| Seta 9-IV-VI* | Anterior to seta 8 | Posterior to seta 8 | Posterior to seta 8 |
| Paddle* | Apex slightly concave | Apex produced | Apex longer on mesal side |
| Fourth-instar larvae |  |  |  |
| Seta 4-C* | Short, 7-11 branches | Short, 3-6 branches | Long, multibranched |
| Setae 5,6,8-C | Single | Single | Multiple branches |
| Seta 13-C | Single | Single | Branched |
| Seta 14-C* | Branched | Single | Branched |
| Cervical sclerite | Present | Present | Absent |
| Seta 1-P* | Shorter than 2-P | Longer than 2-P | Shorter than 2-P |
| Setae 1,3-P* | Stellate | Single | Stellate |
| Seta 5-P* | Double | Single | Multibranched |
| Seta 5-T* | Large, stellate, multibranched | Small, single | Small, branched |
| Seta 2-I-VII* | Large, stellate, far anterolateral to seta 1 | Small, single, near seta 1 | Large, stellate, far anteromesal to seta 1 |
| Seta 11-I* | Large, stellate | Small, 1-2 branches | Large, stellate |
| Seta 5-II-VI* | Large, stellate | Small, single | Large, stellate |
| Seta 7-II* | Short, stellate | Long, 1-2 branches | Short, stellate |
| Seta 9-II-VI* | Far anterior to seta 7 | Near seta 7 | Near seta 7 |

Table 1. Continued

| Character | Borichinda | Isoaedes | Ayurakitia |
| :--- | :--- | :--- | :--- |
| Seta 10-II-V* | Mesal to setae 11,12 | Lateral to setae 11,12 | Lateral to setae 11,12 |
| Seta 13-III-V* | Stellate | Single | Stellate |
| Seta 5-VII* | Large, stellate | Small, 1-2 branches | Large, stellate |
| Comb scales* | Spinelike, in single row | Scalelike, in patch | Spinelike, in single row |
| Siphon acus | Present | Present | Absent |
| Pecten spines* | Evenly spaced | Distal spine more | Evenly spaced |
|  |  | widely spaced <br> Seta 1-X* | Large, branched |
| Precratal setae* | Present | Absent |  |

clearly support the recognition of Borichinda as a new polythetically diagnosed genus of tribe Aedini.

Despite the results of the cladistic analyses, the affinities of Borichinda are somewhat enigmatic. The fourth-instar larvae resemble some species of Stegomyia with stellate setae and precratal setae (e.g., the eastern Palaearctic St. chemulpoensis Yamada), but exhibits significant differences in adult ornamentation (e.g., scutal scaling and leg markings), male genitalia (e.g., development of the claspette and paraproct) and pupal chaetotaxy (e.g., placement of seta 6-VII relative to 9-VII). Similar types of differences also distinguish the adult, larval, and pupal stages of Borichinda from all other genera of the Aedes genusgroup (Reinert et al. 2004) in Southeast Asia, which, in addition to Ayurakitia, Isoaedes and Stegomyia, include Aedimorphus, Alanstonea, Armigeres, Belkinius, Bothaella, Diceromyia, Edwardsaedes, Heizmannia, Lorrainea, Paraedes, Scutomyia, Udaya, Verrallina, and Zeugnomyia.

## Borichinda cavernicola Rattanarithikul and Harbach, new species

(Figs. 1-4)
Female.-As described for genus. Dark scaling dark brown to black, pale scaling white. Head: Dorsum with narrow pale decumbent scales that become broader anteriorly and especially laterally, with narrow pale to dark brown
erect forked scales that become sparser anteriorly. Antenna length 1.761.97 mm (mean 1.86 mm ); pedicel and flagellomere 1 with small pale spatulate scales on mesal surface. Proboscis (except labella) and maxillary palpus darkscaled; proboscis length $1.78-2.03 \mathrm{~mm}$ (mean 1.88 mm ), essentially same length as forefemur; maxillary palpus bare beneath, with relatively few setae dorsally, laterally and apically, length 0.28 0.43 mm (mean 0.37 mm ). Thorax: Integument dark brown, sutures, membranes, lower mesokatepimeron, metapleuron and metameron paler. Scutum with pattern of coarser pale scales on background of finer dark scales, pale scaling as follows: narrow acrostichal line bifurcating into lateral prescutellar lines, patch anteriorly on scutal fossa and dorsocentral area, posterior fossal line bifurcating into posterior dorsocentral and supraalar lines that converge posteriorly, small patch on antealar area; pleural setae as follow: 4-6(6) upper proepisternal, 7-12(10) antepronotal, 2-4(3) postpronotal, 4-6(5) postspiracular, 5-8(6) prealar, total of 8,9 upper and lower mesokatepisternal in continuous line above lower mesokatepisternal scale patch, $1,2(1)$ small lower mesokatepisternal below patch, 2,3(2) anterior mesepimeral, 4-7(5) upper mesepimeral. Wing: Length $2.84-3.46 \mathrm{~mm}$ (mean 3.13 mm ). Halter: Integument pale, scabellum and capitellum with pale scales. Legs: Anterolateral surface of

forecoxa with pale spatulate scales and prominent dark setae, midcoxa with pale spatulate scales on anterior side of mid-lateral row of prominent setae, hindcoxa without scales, with posterolateral row of prominent setae; ventral surface of trochanters with pale scales and setae distally; femora with distinct apical pale rings and subdued posterolateral pale stripes on proximal $0.5-0.8$; forefemur length $1.81-1.03 \mathrm{~mm}$ (mean 1.90 mm ). Abdomen: Basal pale bands become obsolescent medially on segments V-VII in females and usually on segment VII in males; sterna progressively more dark-scaled from segment II to segment VII, sterna VI,VII and sometimes VI entirely dark-scaled. Genitalia (Fig. 1H-K): Tergum VIII broad anteriorly, narrower posteriorly, anterior margin slightly convex, index about 0.65 ; sternum VIII narrow anteriorly, broadened laterally in distal 0.6 , index about 0.55 , with close-set line of setae on either side of posterior emargination; tergum IX index about 0.70; upper and lower vaginal lips narrow, lightly to moderately pigmented; postgenital lobe (PGL) with lateral margins slightly depressed, dorsal index about 0.88 , ventral index about 1.25 , ventral length about 0.1 mm ; cercus with smoothly rounded apex, distal area of dorsal and lateral surfaces with scattered setae, length about 0.18 mm , width about 0.06 mm , index about 3.0, cercus/dorsal PGL index about 2.5 ; spermathecal capsules (not illustrated) heavily pigmented, with few small pores near orifice.

Male.-Smaller but otherwise similar to female except for obvious sexual differences. Head: Antenna with strongly
developed whorls of numerous long setae, 2 terminal flagellomeres disproportionately long compared to other flagellomeres; length $1.27-1.43 \mathrm{~mm}$ (mean 1.37 mm ). Proboscis proportionately shorter, length $1.67-1.84 \mathrm{~mm}$ (mean 1.77 mm ), very slightly shorter to same length as forefemur, proximal 0.75 of ventral surface with subdued and indistinct pale scaling. Maxillary palpus slightly shorter than proboscis, length $1.62-1.78 \mathrm{~mm}$ (mean 1.70 mm ), darkscaled, palpomeres $1-3$ without scales ventrally, apices of palpomeres $3-5$ with few relatively short rather inconspicuous setae, palpomeres 4 and 5 short and bent laterad. Wing: Generally paler, veins with fewer scales; length $2.57-2.73 \mathrm{~mm}$ (mean 2.66 mm ). Abdomen: Tergum VIII with dark scales dorsally, pale-scaled laterally; sternum VIII pale-scaled. Genitalia (Fig. 1A-G): Gonocoxite with patch of long mesally projecting setae on distal half of tergomesal margin, some more distal setae flattened and lanceolate; dorsal surface with short setae, lateral and ventral surfaces with long setae and spatulate scales; gonostylus moderately long and narrow, slightly longer than half length of gonocoxite, tergomesal surface with scattered setae beyond midlength, gonostylar claw shoe-horn-shaped; basal mesal lobe with flattened tergally directed head bearing numerous long close-set caudally projecting setae; proctiger nearly half as long as gonocoxite, paraproct distally tapered and bend tergally, without apical teeth; tergum X poorly defined, fused caudally with base of paraproct; aedeagus with lateral plates bent tergally, joined distally, tergolateral margin of
$\leftarrow$
Fig. I. Male (A-G) and female (H-K) genital structures of Borichinda cavernicola. Aspects as indicated. Scales in mm . $\mathrm{Ae}=$ aedeagus; $\mathrm{BML}=$ basal mesal lobe; $\mathrm{Ce}=$ cercus; $\mathrm{Gc}=$ gonocoxite; $\mathrm{Gs}=$ gonostylus; $\mathrm{I}=$ insula; LVL $=$ lower vaginal lip; Par $=$ paramere; $\mathrm{PGL}=$ postgenital lobe; $\mathrm{Ppr}=$ paraproct; $\mathrm{Pr}=$ proctiger; UVL $=$ upper vagina lip; VIII-S $=$ sternum VIII; VIII-Te $=$ tergum VIII; IX-S $=$ sternum IX; IX-Te $=$ tergum IX; X-Te $=$ tergum $X ; 1-3-S=$ numbered setae of sternum VIII.

Table 2. Range (mode) of numbers of branches for pupal setae of Borichinda cavernicola.

|  | $\begin{array}{c}\text { Cephatothorax } \\ \text { Seta }\end{array}$ | $\begin{array}{c}\text { CT }\end{array}$ | I | II | III | IV | V | VI | VII | VIII |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | \(\left.\begin{array}{c}Paddle <br>

Pa\end{array}\right]\)
each lined with short teeth to apex; paramere moderately long, about 0.7 length of aedeagus; basal piece large, broadly sickle-shaped.

Pupa (Fig. 2A,B).-As described for genus; character and positions of setae as illustrated, numbers of branches in Table 2. Cephalothorax: Lightly to moderately pigmented, scutum slightly darker, becoming progressively darker onto metanotum. Setae $1-3,5-7,9-\mathrm{CT}$ single, $3,5,7-\mathrm{CT}$ rarely double; 4-CT usually double, rarely single; 8-CT double or triple, rarely single; 11-CT single or double more often single, noticeably aciculate distally. Trumpet: Moderately and evenly pigmented; length 0.330.41 mm (mean 0.41 mm ), tracheoid $0.03-0.05 \mathrm{~mm}$ (mean 0.04 mm ), pinna $0.04-0.09 \mathrm{~mm}$ (mean 0.07 mm ), width at midlength $0.06-0.08 \mathrm{~mm}$ (mean 0.07 mm ), index 4.13-7.50 (mean 5.54). Abdomen: Moderately pigmented, posterior area of tergum I darker, posterior areas of terga and sterna II-VII and their fold lines darker; length $2.96-3.68 \mathrm{~mm}$ (mean 3.19 mm ). Seta 1-I bushlike, with thick stem and numerous (approximately 120) thin branches; seta 1-II-VII multiple branched, with progressively fewer branches on succeeding posterior terga;

6-II-VI relatively long, single, occasionally double on segments II and III; 10-II long, single (apparently homologous with short seta in same position on other aedine species interpreted as seta 11); normally double, branches usually noticeably aciculate distally, 9-VIII with $4-$ 7(5) long aciculate branches, slightly longer than half length of paddle. Genital lobe: Moderately pigmented; length $0.45-0.47 \mathrm{~mm}$ in male; $0.24-0.27 \mathrm{~mm}$ in female. Paddle: Lightly and evenly pigmented, margins and midrib slightly darker, midrib distinct to distal area before seta $1-\mathrm{Pa}$; length $0.67-0.82 \mathrm{~mm}$ (mean 0.75 mm ), width $0.41-0.55 \mathrm{~mm}$ (mean 0.50 mm ), index 1.39-1.63 (mean 1.49).

Larva, fourth-instar (Fig. 3).-As described for genus; form and placement of setae as figured, range and modal number of branches in Table 3. Head: Nearly round in dorsal view, length $0.63-$ 0.88 mm (mean 0.81 mm ), width $0.72-$ 0.76 mm (mean 0.76 mm ); moderately pigmented, collar darker. Dorsomentum with 10 or 11 teeth on either side of larger median tooth. Setae $14-\mathrm{C}$ and 6 Mx with thick stiff branches; 18-C single, inserted posterior to cervical sclerite. Antenna: Moderately to darkly pigmen-
Table 3. Range (mode) of numbers of branches for fourth-instar larval setae of Borichinda cavernicola.

| Seta | Head | Thorax |  |  | Abdominal Segments |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | C | P | M | T | 1 | II | III | IV | V | VI | VII | VIII | X |
| 0 | 1 | 6-10(7) | - | - | - | 1 | 1 | 1 | 1 | 1 | 1 | 1 | - |
| 1 | 1 | 3-5(5) | 5-10(6) | 9-14(12) | 11-15(13) | 9-13(10) | 8-11(9) | 5-11(9) | 7-10(9) | 8-12(8) | 6-9(8) | 4-6(5) | 3,4(3) |
| 2 | - | 1 | 1 | 1 | 10-14(11) | 6-12(10) | 7-12(10) | 5-12(8) | 6-12(9) | 6-10(7) | 5-9(7) | 1 | 3-5(4) |
| 3 | 1 | 5-12(7) | 1 | 1-3(2) | 1,2(1) | 1 | 1 | 1 | , | ( | 1 | 4.7(5) | 1,2(1) |
| 4 | 7-11(9) | 1 | 1 | 3-5(4) | 2,3(3) | 1-3(2) | 1 | 1,2(1) | 1-3(2) | 1 | 1 | 1,2(1) | 1-4* |
| 5 | 1 | 2 | 3-5(3) | 6-12(10) | 4-8(6) | 8-12(10) | 7-13(10) | 8-13(11) | 6-11(9) | 6-12(9) | 6-9(6) | 5-8(6) | - |
| 6 | 1 | 1,2(1) | 1 | 1 | 3-6(5) | 3-6(5) | 3-6(4) | 2-5(3) | 2,3(3) | 2,3(3) | 3-6(4) | - | - |
| 7 | 5-8(7) | 2,3(2) | 2-4(3) | 2-6(4) | 2-4(2) | 4-6(4) | 5-8(6) | 4 8(6) | 5-10(6) | 3-5(4) | 1 | 1-S, | 2-4(4) |
| 8 | 1 | 5-13(9) | 4-7(5) | 5-11(6) | - | 1 | , | 1 | 1 | 2 | 5-7(5) | 2-S, | 1 |
| 9 | 1,2(1) | 1,2(1) | 1 | 4-6(5) | 2-4(3) | 4-8(6) | 6-12(8) | 6-12(8) | 5-10(8) | 6-8(8) | 4-6(4) | 6-S, | 1 |
| 10 | 1 | 1 | , | 1,2(1) | 1 | 1 | 1,2(1) | 1 | 1 | 1 | 1,2(1) | 7-S, | 1 |
| 11 | 6-11(7) | 1,2(2) | 1 | 1,2(1) | 8-15(8) | 1 | 1 | 1 | 1 | 1 | 1 | 8-S, | 1 |
| 12 | 1,2(2) | 1 | 5-10(7) | 1 | - | 1,2(1) | 1,2(1) | 1 | 1,2(1) | 1 | 1 | 9-S, | 1 |
| 13 | 1 | - | 6-10(7) | 6-12(11) | 8-11(8) | 6-9(8) | 6-8(6) | 5-7(6) | 5-8(6) | 7-10(8) | 5-10(9) | - | - |
| 14 | 3-6(4) | 6-9(8) | 4-8(6) | - | - | - | 1 | 1 | 1 | 1 | 1 | 1 | - |
| 15 | 1,2(1) | - | - | - | - | - | - | - | - | - | - | - | - |
| 18 | 1 | - | - | - | - | - | - | - | - | - | - | - | - |

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ted; length $0.28-0.38 \mathrm{~mm}$ (mean 0.34 mm ). Thorax: Integument hyaline, smooth. Setae 3-P and 1-T minutely aciculate; $5-7-\mathrm{P}, \mathrm{M}, \quad 9-\mathrm{M}, \mathrm{T}$ and $7-\mathrm{T}$ aciculate proximally; 5,7-P double, 7-P rarely triple; 6-P single or double, more often single; 14-P relatively strongly developed, multiple branched, similar to $14-\mathrm{M}$; 5-T also relatively strongly developed, multiple branched, similar to 8-T. Abdomen: Integument hyaline, smooth. Seta 3-I usually and 3-II-VII always single, moderately long, 3-I-V noticeably longer than seta 4, 3-VI,VII subequal to seta 4 ; 6-I-VI multiple branched, branches long, stout, aciculate proximally, 6-VI noticeably shorter than $6-\mathrm{I}-\mathrm{V} ; 8$-II-V single, $8-\mathrm{VI}$ double; 10-I-VII normally single, moderately long, $10-\mathrm{II}-\mathrm{V}$ approach length of following segment. Segment VIII: Comb comprised of $8-12(10)$ spinelike scales with minute basolateral denticles. Siphon: Moderately to darkly pigmented, surface with minute rows of scalelike etching; length $0.70-0.83 \mathrm{~mm}$ (mean 0.78 mm ); width at base $0.21-0.30 \mathrm{~mm}$ (mean 0.26 mm ); index 2.50-3.33 (mean 3.00 ); pecten with $9-16(13)$ spines with few denticles basally on ventral margin. Segment $X$ : Saddle moderately pigmented; dorsal length 0.240 .28 mm (mean 0.27 mm ); siphon/saddle index 2.88-3.08 (mean 2.95). Seta 1-X usually with 3, occasionally 4 , stout rather stiff branches that diverge in single plane from base; 2 precratal setae of ventral brush stellate with short stiff branches. Dorsal and ventral anal papillae equally long, longer than saddle, length $0.31-0.53 \mathrm{~mm}$ (mean 0.45 mm ), thick, abruptly tapered apically.

Larva, third-instar (Fig. 2C).-Like fourth-instar but smaller; large setae generally with slightly fewer branches; comb scales shorter, broader, in partially double row; acus larger, attached to base of siphon; spines on posterior margin of saddle larger, especially prominent dorsally; precratal setae of ventral brush absent.

DNA sequence.-The ITS2 and COI sequences generated during the study are available in GenBank under the following accession numbers: ITS2 (EF370409EF307411) and COI (EF370412, EF370413). Considering the taxonomic breadth of Aedini, DNA sequence data are available for very few species of the tribe, which precludes comparisons with allied taxa. Among sequences recorded in GenGank, the 393-bp ITS2 fragment of Bc. cavernicola, which includes a short portion of the flanking 5.8 S and 28 S genes, is most similar ( $84.4 \%$ ) to that of Stegomyia simpsoni (AF158208, registered as Aedes simpsoni). Thus, of the species available for comparison, Bc. cavernicola and St. simpsoni are the most closely, albeit distantly, related species. The nucleotide bases of the ITS2 fragment consist of $24.4 \% \mathrm{~A}, 25.7 \% \mathrm{~T}$, $26.5 \% \mathrm{C}$ and $23.4 \% \mathrm{G}$. No intraspecific variation was detected between the ITS2 sequences generated for the three larvae, and only two unique mtDNA haplotypes were detected in the 522-bp COI fragment, which varied by only a singleton polymorphism at base $459(\mathrm{C} \leftrightarrow \mathrm{T})$ in one specimen. The most common COI haplotype comprised $28.1 \% \mathrm{~A}, 37.5 \% \mathrm{~T}$, $19.1 \% \mathrm{C}$ and $15.3 \% \mathrm{G}$. Although based on a small sample size $(n=3)$, this level of genetic homogeneity denotes a closely

Fig. 2. A, B, Pupa of Borichinda cavernicola. A, Left side of cephalothorax, dorsal to right. B, Dorsal (left) and ventral (right) aspects of metathorax and abdomen. C, Terminal abdominal segments of thirdinstar larva. Scales in mm. $\mathrm{CS}=$ comb scale; $\mathrm{CT}=$ cephalothorax; $\mathrm{GL}=$ genital lobe; $\mathrm{Pa}=$ paddle; $\mathrm{Ps}=$ pecten spine; $\mathrm{S}=$ siphon; $\mathrm{Sa}=$ saddle; $\mathrm{T}=$ trumpet; $\mathrm{I}-\mathrm{VIII}, \mathrm{X}=$ abdominal segments $\mathrm{I}-\mathrm{VIII}, \mathrm{X} ; 0-\mathrm{I} 1,14$ $=$ setal numbers for specified areas, e.g., seta 3-I.



Fig. 4. Rimstone pool in Borichinda Cave - the larval habitat and type locality of Borichinda cavernicola. The pool is fed by seepage from a single point (arrow) in the wall of the cave.
related, potentially inbreeding population. Among sequences available in GenBank, the COI gene of Bc. cavernicola proved to be most similar ( $87.4 \%$ ) to that of Anopheles funestus (AY423059), which tells us nothing about the affinities of Borichinda.

Etymology.-The name cavernicola is a Latin common noun (masculine or feminine) meaning "cave-dweller." It was chosen because the species was discovered in and only known to inhabit

## Borichinda Cave in northern Thailand

 (see below).Bionomics.-Larvae and pupae of $B C$. cavernicola were collected on three occasions from a single rimstone pool (Fig. 4) located approximately 150 m into Borichinda Cave. Other than limited areas of seepage, no other sources of water were found in the cave. The collections were made in October 2004, September 2005, and November 2005 during the latter part of the rainy season. The water in the

Fig. 3. Fourth-instar larva of Borichinda cavernicola. A, Head, dorsal (left) and ventral (right) aspects of left side. B, Thorax and abdominal segments I-VI, dorsal (left) and ventral (right) aspects of left side. C, Abdominal segments VII-X, left side. Scales in mm. $\mathrm{A}=$ antenna; $\mathrm{C}=$ cranium; $\mathrm{M}=$ mesothorax; $\mathrm{P}=$ prothorax: $\mathrm{S}=$ siphon; $\mathrm{Sa}=$ saddle; $\mathrm{T}=$ metathorax; $\mathrm{I}-\mathrm{VIII}, \mathrm{X}=$ abdominal segments $\mathrm{I}-\mathrm{VIII}, \mathrm{X} ; 0-15,18$ $=$ setal numbers for specified areas, e.g., seta $5-\mathrm{C}$.
pool was crystal clear, $20-30 \mathrm{~cm}$ deep and devoid of vegetation and plant matter. A layer of silt covered the bottom of the pool. There was no water in the pool when the cave was visited in February 2005, but the silt bottom was slightly moist. Samples of silt were collected and later inundated with water, but eggs were apparently absent as no larvae emerged. The walls of the cave were searched for resting adults, but none were found. The cave harbours an enormous number of bats that are likely to be the primary source, perhaps the sole source, of food for adult females. The floor of the cave was covered with bat droppings, but none were observed in the rimstone pool. Since the rimstone pool appears to be the only larval mosquito habitat in Borichinda Cave, and in view of the very long period of time it takes the wall of a rimstone pool to develop, it is hypothesized that $B C$. cavernicola evolved as countless generations developed in the same habitat.

Distribution.-Borichinda cavernicola is only known from Borichinda Cave in Chiangmai Province in northern Thailand.

Type series.-One hundred and seventy-four specimens: 38 i, 16 §, $45 \mathrm{Le}, 51 \mathrm{Pe}, 20 \mathrm{~L} 4,4 \mathrm{~L}$. Holotype, \$ (TH122-25), with LePe and dissected genitalia on separate microscope slides, THAILAND: Chiangmai Province, Chomthong District, Borichinda Cave ( $18^{\circ} 29^{\prime} 59.5^{\prime \prime} \mathrm{N} 98^{\circ} 40^{\prime}$ $40^{\prime \prime} \mathrm{E}$, on shared border of Doi Inthanon and Mae Wang National Parks), rimstone pool, 13.x. 2004 (Rampa et al.). Paratypes (same data as holotype except collection TH343 collected 29.ix. 2005 by Harbach et al.; specimens with dissected genitalia are indicated with an asterisk*), 1 ㅇ (TH122-11); 32 ${ }^{\circ} \mathrm{CePe}$ (TH122-4, $-8,-9,-10,-12,-13$, $-15,-16,-17,-18,-19,-20^{*},-21,-22,-23$, $-24,-26,-27,-28$; TH343-1b, $-2,-4,-7$, $-11,-12,-15,-16,-18,-20,-22,-24,-26)$;

1 ¢ Le (TH343-14*); 4 ¢Pe (TH122-1*, -2, -3; TH343-3); 1 के (TH343-19); 11 के LePe (TH122-4*, -6*; TH343-1a*, -5, -6, $-9,-10,-13,-17,-21,-23) ; 3$ के Pe (TH3435, -8, -25*); $20 \mathrm{~L}^{4}$ (TH122a, b, c, d; TH343e-t); 4 L $^{3}$ (TH343a, b, c, d).

The holotype (TH122-25) and the following paratypes are deposited in the NHM: TH 122-1 through -10, -12 through $-15,-18$ through -28 . The remaining paratypes are deposited in the Queen Sirikit Botanic Garden, Chiangmai, Thailand (TH122-11, -16, -17; all of collection TH343).

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## Appendix

Anatomical characters used in the cladistic analysis. See Reinert et al. (2004) for character coding and discussions of the characters. Character states observed in Borichinda cavernicola are listed with the numerical character code of Reinert et al. in parentheses.

## Eggs

1. Deposition: unknown (?).
2. Shape: unknown (?).
3. Outer chorion: unknown (?).

## Larvae (fourth-instars)

4. Labiogula: short, length $<$ width (0).
5. Antenna: moderate to long, $\geq 0.42$ median length of dorsal apotome (1).
6. Seta 1-A, development: single or 2branched (0).
7. Seta $I-A$, length: short, $\leq 3.0 \times$ antennal width at point of attachment (0).
8. Setae 2,3-A, position: apical or nearly apical (0).
9. Seta 1-C, development: single, thinner, distal part thin (1).
10. Seta 4-C, position: at same level or posterior to seta 6-C (1).
11. Seta 4-C, length: moderate to long (1).
12. Seta $6-C$, position: anterior to seta $7-$ C (0).
13. Seta 7-C, position: anterior to seta 5C (0).
14. Seta $12-C$, position: mesad of or at same level as seta 13-C (0).
15. Seta $13-C$, development: single (0).
16. Seta 19-C: absent (0).
17. Ventromedian cervical sclerite: present (1).
18. Setae 1-3-P: not attached to a common setal support plate (0).
19. Seta $1-P$, length: $\leq$ length of seta $2-\mathrm{P}$ (0).
20. Seta 3-P, length: $<$ length of seta $2-\mathrm{P}$ (0).
21. Seta $5-P$, length: $\leq$ length of seta 6-P (0).
22. Seta 5-P, development: branched (1).
23. Seta $8-P$, length: $\leq 1.2$ length of seta 4-P (0).
24. Seta 13-P: absent (0).
25. Seta 4-M, development: single (0).
26. Seta $7-M$, length: $<$ length of seta 5M (0).
27. Seta 2-T, development: single (0).
28. Seta 3-I, development: single (0).
29. Seta $7-I$, length $: \geq 0.55$ length of seta 6-I (1).
30. Seta $7-I$, development: single to $\geq 4$ branches $(0,1)$.
31. Seta 12-I: absent (0).
32. Seta 6-II, development: branched (1).
33. Seta 7-II, development: different than seta 7-I (1).
34. Seta 8-II, development: single (0).
35. Seta $3-V$, length: $\geq 1.90 \times$ length of seta $5-\mathrm{V}$ (1).
36. Seta 2-VII, development: branched (1).
37. Seta 3-VII, length: short to moderately long (0).
38. Seta 12-VII, position: posterior to seta 13-VII (2).
39. Seta 12-VII, development: single (0).
40. Setae 1.2-VIII: not attached to common setal support plate (0).
41. Seta 2-VIII, development: single (0).
42. Seta 4-VIII, development: single (0).
43. Comb: few to several scales in 1 or 2 irregular rows (0).
44. Comb plate: absent (0).
45. Siphon, acus: present (1).
46. Pecten: present, spines evenly spaced (1).
47. Seta $1-S$ : one seta some distance from base of siphon (1).
48. Seta $6-S$, development: short (1).
49. Seta 8-S, lengtli: short (0).
50. Saddle, acus: absent (0).
51. Sclerotization of segment $X$ : dorsal saddle (0).
52. Seta 1-X, position: inserted on saddle (0).
53. Seta 3-X, development: single (0).
54. Sclerotization supporting seta $4-X$ (ventral bush): grid with only transverse bars (1).
55. Precratallpreboss setae (i.e., 2 or more setae anterior to grid/boss): present (1).
56. Seta $4 a-X$ of ventral brush: long (1).

## Pupae

57. Cephalothorax with clear unpigmented spots: absent (0).
58. Tracheoid area of trumpet: present at base, weakly developed (1).
59. Seta $1-C T$, development: similar in development to seta 3-CT (0).
60. Seta $5-C T$, length: $>1.3 \times$ length of seta 4-CT (1).
61. Seta $7-C T$, length: $\leq$ length of seta $6-$ CT (0).
62. Seta 11-CT, development: single or branched $(0,1)$.
63. Seta 13-CT: absent (0).
64. Seta $6-I$, length: $\leq$ length of seta $7-\mathrm{I}$ (0).
65. Seta 2-II, position: lateral to seta 1-II (1).
66. Seta 2-II, length: < length of seta 1II (0).
67. Seta 3-II, position: lateral to seta 2-II (1).
68. Seta $3-$ II, length: $\leq$ length of seta 6 -II (0).
69. Seta 3-III, development: single, stout (0).
70. Seta 5-II, position: lateral to or at same level as seta 4-II (0).
71. Seta $6-$ II, length: $>$ length of seta $7-$ II (1).
72. Seta 6-III, development: single (0).
73. Seta 6-VII, position: anterior to seta 9-VII (0).
74. Seta $5-V$, length: $\geq$ medial length of tergum VI (1).
75. Seta 2-VI, position: lateral to seta 1VI (1).
76. Seta 3-VI, position: lateral to seta 1VI (1).
77. Seta 9-VII, length: $>$ length of seta 6-VII (1).
78. Seta 9-VIII, development: $\geq 3$ branches (1).
79. Paddle midrib: well developed, extending to or near apex of paddle (1).
80. Paddle, fringe of long hairlike spicules: absent (0).
81. Paddle, apical margin: sharply or broadly rounded, flat or very slightly concave (0).
82. Seta 1-Pa, development: single, rarely 2-branched (0).
83. Seta 2-Pa: absent (0).

Adults (females except where otherwise noted)
84. Erect scales of head: on occiput and vertex (2).
85. Decumbent scales of vertex: narrow (1).
86. Ocular line: narrow (0).
87. Ocular scales: narrow (1).
88. Eyes, immediately above antennal pedicels: narrowly to moderately separated (1).
89. Interocular setae: present (1).
90. Interocular space, scales: narrow (1).
91. Antennal pedicel, mesal surface: with scattered scales and/or setae (0).
92. Antennal pedicel, lateral surface: bare (0).
93. Apical flagellomeres (males): both apical flagellomeres disproportionately long compared with other flagellomeres (0).
94. Antenna, development of flagellar whorls (males): numerous long setae, directed normally dorsally and ventrally (2).
95. Maxillary palpomeres (males): 5, palpomeres 2 and 3 fused/ankylosed (0).
96. Antepronota: more widely separated (1).
97. Acrostichal setae: present (1).
98. Dorsocentral setae: present (1).
99. Prescutellar area, median andlor posterior parts: bare (0).
100. Prescutellar setae: present (1).
101.Scutellum, scales on midlobe: broad (1).
102.Scutellum, scales on lateral lobes: narrow (0).
103. Paratergal scales: absent (0).
104.Parascutellar scales: absent (0).
105. Antepronotal scales: present (1).
106. Postpronotal scales: present (1).
107. Prespiracular setae: absent (0).
108. Postspiracular setae: present (1).
109. Postspiracular scales: present (1).
110. Hypostigmal scales: absent (0).
111.Subspiracular area: with scales and/ or setae (1).
112. Upper proepisternal setae: 5-19 (1).
113. Upper proepisternum, scales: present (1).
114. Lower proepisternum, scales: absent (0).
115. Upper mesokatepisternal setae: present (1).
116. Prealar setae: $\leq 20$ (0).
117.Lower prealar area, scales: absent (0).
118. Upper prealar area, scales: absent (0).
119. Mesepimeron, scales: present (1).
120.Lower anterior mesepineral setae: present (1).
121.Mesepimeron, fine setae on ventral, posterior or both areas: absent (0).
122. Metameron: bare (0).
123. Upper calypter, setae or hairlike scales: present, several or numerous (1).
124. Upper calypter, setae or hairlike scales (males): present, several to numerous (1).
125. Alula, marginal scales: narrow (1).
126.Alula, dorsal moderately broad or broad scales: absent (0).
127. Remigial setae, dorsal: absent (0).
128. Remigial setae, ventral: absent (0).
129. Anal vein of wing: ending noticeably distal to intersection of mcu and cubitus (1).
130. Vein $R_{2}$, length: $\geq$ length of vein $\mathrm{R}_{2+3}$ (1).
131. Anteprocoxal scales: absent (0).
132. Postprocoxal scales: absent (0).
133. Base of hindcoxa: well below dorsal margin of mesomeron (0).
134. Fore-, mid- and hindfemora, subapical white-scaled band: absent (0).
135.Hindtarsomere 1, pale-scaled area: absent (0).
136.Foreungues: both toothed (2).
137.Foreungues (males): one simple, other toothed (1).
138. Midungues (males): both toothed (2).
139.Hindungues (males): both simple (0).
140. Hindungues: both simple (0).
141.Laterotergite, scales: present (1).
142. Terga, lateral setae (males): numerous, short to moderately long (1).
143. Abdominal segment VII, shape: laterally compressed (0).

## Female genitalia

144. Intersegmental membrane between segments VII and VIII: intermediate (1).
145.Tergum VIII: entirely sclerotized (rarely with only small median nonsclerotized area), without lateral rod-shaped structures (2).
145. Tergum VIII, setae: on distal 0.6 or less (0).
147.Tergum VIII, scales: numerous, $\geq 9$ scales (2).
146. Sternum VIII, position of seta 2-S: noticeably posterior to seta 1-S (0).
147. Sternum VIII, scales: numerous, $\geq 10$ scales (2).
150.Tergum IX: narrower, width $\leq 1.9 \times$ length (1).
148. Tergum $I X$, setae: present distally (1).
149. Postgenital lobe, position of ventral setae: distal (0).
150. Upper vaginal sclerite: present (1).
151. Lower vaginal sclerite: absent (0).
152. Insula: tonguelike (2).
156.Insular setae: absent (0).
157.Spermathecal capsule(s): 3 (1).

## Male genitalia

158. Tergum IX, setae: slender (1).
159. Sternum $I X$, length: long (2).
160.Stermum IX, setae: present (1).
161.Gonocoxite, lateral setae: mostly long (1).
162.Gonocoxite, mesal surface: entirely membranous (0).
160. Gonocoxite, scales: present (1).
161. Gonostylus, attachment to gonocoxite: apical (1).
165.Gonostylus, scales: absent (0).
162. Gonostylus, development: moderately long to long, relatively narrow throughout length but distal part narrower and usually somewhat curved mesally (0).
163. Gonostylar claw: one (1).
164. Claspette: with basal, setose plaque variously developed (setae simple, specialized or both) or with relatively short, thin or thick stem with slender or stout seta(e) or spiniform(s) (1).
165. Opisthophallus: absent (0).
170.Aedeagus: comprised of 2 lateral plates (1).
166. Proctiger, sternal arm: absent (0).
167. Proctiger, cercal setae: absent (0).

[^0]:    * Cratal setae: anterior setae normally single, rarely double; posterior seta 3- or 4-branched. Posterior precratal seta with 2-4(4) branches; anterior precratal seta with $2,3(3)$ branches.

